FOODBORNE PATHOGENS AND DISEASE
Volume 6, Number 1, 2009

DOI: 10.1089/fpd.2008.0153

Evaluation of a Multiplex PCR System for Simultaneous Detection of *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7 in Foods and in Food Subjected to Freezing

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Abstract

Conventional culture methods were compared to a multiplex polymerase chain reaction (PCR) assay for simultaneous detection of *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7 from enrichment cultures of various types of artificially inoculated and naturally contaminated foods. The multiplex PCR assay was evaluated in 44 types of spiked food samples, including meat, produce, fish, and dairy products targeting genes specific for each pathogen for simultaneous detection. The sensitivity of the assay was ≤5 CFU/25 g of inoculated sample after 20 hours of enrichment. The PCR assay was also evaluated in inoculated food samples stored at −20°C for 2 weeks or 2 months. Out of 28 food samples tested, 27, 27, and 26 samples were positive for *Salmonella* Enteritidis, *L. monocytogenes*, and *E. coli* O157:H7, respectively, using the multiplex PCR assay, whereas only 13, 26, and 20 samples were positive, respectively, using the culture method after 2 weeks of storage at −20°C. Similar results were obtained for samples stored at −20°C for 2 months. The multiplex PCR assay method was capable of detecting 5 colony-forming units of each of the three pathogens per 25 g of more than 40 types of food, and the detection rate of the PCR assay was higher than that of conventional culture methods. As a result, the multiplex PCR assay is a valuable method for simultaneous rapid screening for the three pathogens in food, even after frozen storage.

Introduction

Foodborne illness caused by *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7 is a major public health concern worldwide (McLauchlin *et al*., 1996; Schlosser *et al*., 2000; Wells *et al*., 2004). There are approximately 1.4 million cases of illness annually, resulting in 1000 deaths (Mead *et al*., 1999; Buzby, 2000). The availability of reliable detection methods is critical for identifying the pathogenic bacteria in foods, food ingredients, and food processing plants. Because conventional culture methods for detecting pathogens are time consuming, results are frequently not available until the food has been either released to the market or consumed, thus increasing the risk of pathogen transmission. Furthermore, pathogens are often present in food in very low numbers with a high background of indigenous

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microflora, thus rendering the recovery of target organisms difficult.

Rapid and sensitive assays with high specificity are needed for detecting pathogenic bacteria in foods and other types of samples, and polymerase chain reaction-(PCR) based methods meet these requirements. Since conventional culture methods generally require the use of media containing selective agents for pathogen detection and isolation, it may be difficult to detect injured cells that have the potential to recover and grow when the food is consumed. The use of genetic-based techniques enables the detection of pathogenic microbes exposed to stress conditions with greater sensitivity and reliability than conventional culture methods. For PCR detection, the enrichment medium does not require the use of selective agents, thus allowing detection of both healthy and injured cells (Uyttendaele *et al*., 1998). Therefore, screening using PCR methods can increase the detection rate of pathogenic bacteria. There have been many reports on pathogen detection using PCR methods evaluated in various foods, such as chicken, milk, ground beef, and others (Thomas *et al*., 1991; Croci *et al*., 2004). However, many of these reports described detection from pure cultures or from a specific food matrix. There have been few reports describing sample treatments conducted prior to the PCR to remove PCR inhibitors from a variety of food matrices, followed by detection of pathogenic bacteria exposed to stress conditions, such as frozen storage.

Real-time PCR assays employing various types of fluorescence systems allow direct detection of product without the gel electrophoresis and staining steps (Oberst *et al*., 1998; Kimura *et al*., 1999). Using real-time PCR, the limit of detection is generally in the range of 10^2 to 10^3 colony-forming units (CFU)/mL; however DNA extraction from the food enrichment is still required prior to the PCR. A sensitive, specific, and rapid method that would allow detection of multiple pathogens simultaneously from different types of foods following DNA extraction and PCR amplification would be very valuable for the food industry and regulatory agencies.

We developed a multiplex PCR method capable of determining the presence of *Salmonella* spp., *L. monocytogenes*, and *E. coli* O157:H7 directly from enrichment cultures by targeting the *Salmonella*-specific DNA, *hlyA*, and *eaeA* sequences (Kawasaki 2005). When this method was applied to spiked pork samples containing these pathogens, the detection sensitivity for each pathogen was 1 CFU per 25-g food sample after enrichment for 24 hours. Moreover, excellent agreement between results of the multiplex PCR and the conventional culture method was obtained in naturally contaminated meat samples. In the current study, we evaluated the sensitivity of the multiplex PCR method with various spiked frozen and nonfrozen food samples and with various naturally contaminated foods.

Materials and Methods

Bacterial strains and culture conditions

The bacterial strains used were *Salmonella* Enteritidis IFO3313, *L. monocytogenes* ATCC 49594, and *E. coli* O157:H7 ATCC 43894. Bacterial strains were grown overnight at 37°C with rotary shaking in trypticase soy broth (TSB; BBL, Cockeysville, MD) containing 0.6% yeast extract, unless otherwise stated. For determining the number of viable cells, serial decimal dilutions of cultures in phosphate-buffered saline (PBS) were plated onto trypticase soy agar (TSA; BBL, Cockeysville, MD) for each bacterium. The plates were then incubated at 37°C for 48 hours before enumeration.

Pathogen detection by the conventional culture method

For detection of *Salmonella* spp., each 25-g food sample was mixed with 225 mL of sterile Enterobacteriaceae enrichment mannitol broth (Nissui Seiyaku Ltd., Tokyo, Japan) and pummeled in a Stomacher apparatus for 2 minutes; the mixture was then incubated for 18 hours at 35°C. One milliliter of the culture was added to 10 mL of selenite cystine broth (Eiken Co., Ltd., Tokyo, Japan) and incubated at 43°C for 18 hours. One loopful of the culture was then streaked onto mannitol–lysine–crystal violet–brilliant green agar (Nissui Seiyaku Ltd.) agar and incubated at 35°C for 24 hours. The resulting presumptive *Salmonella* colonies were

subjected to biochemical screening and serological confirmation using *Salmonella* polyvalent O, O1 antisera (Denka Seiken, Tokyo, Japan). For detection of *L. monocytogenes*, 25 g of the food samples were mixed with 225 mL of sterile UVM modified *Listeria* enrichment broth (University of Vermont modified *Listeria* enrichment broth; BD, Cockeysville, MD) and pummeled in a Stomacher for 2 minutes, followed by incubation for 48 hours at 30°C. One loopful of the culture broth was streaked onto polymyxin acriflavine LiCl ceftazidime esculin mannitol agar (Oxoid, Hampshire, UK) and incubated at 35°C for 48 hours. Presumptive colonies were streaked onto horse blood agar and TSA plates and incubated at 35°C for 48 hours. The resulting presumptive *Listeria* colonies were submitted for biochemical screening (oxidase test, catalase test, and Gram staining), and then confirmed with an API-*Listeria* kit (bioMérieux, Marcy l'Etoile, France). For detection of *E. coli* O157:H7, 25 g of the food sample was mixed with 225 mL of sterile mEC medium (Eiken Co., Ltd.) containing novobiocin and pummeled in a Stomacher for 2 minutes; the mixture was then incubated for 18 hours at 42°C. One loopful of the culture broth was streaked onto CHROMagar (Merck, Darmstadt, Germany) and CT-SMAC plates (Nissui Seiyaku Ltd.) and incubated at 35°C for 24 hours. Presumptive *E. coli* O157:H7 colonies were subjected to biochemical screening (cellobiose lactose indole beta-D-glucuronidase agar [Kyokutou seiyaku Co. Ltd., Tokyo, Japan], indole test, triple suger iron agar [Eiken Co., Ltd.], and lysin indole motility agar [Eiken Co., Ltd.]) and serological confirmation using a latex agglutination kit employing antisera against *E. coli* O157 and H7 antigens (*E. coli* O157-AD; Denka Seiken).

Enrichment, DNA extraction, and multiplex PCR conditions

The No17 enrichment medium used for simultaneous growth of *Salmonella* Enteritidis, *L. monocytogenes*, and *E. coli* O157:H7, which was described previously, was used in the current study (Kawasaki *et al.*, 2005). DNA was extracted using the lysis–guanidine isothiocyanate (GuSCN) method also as described previously (Kawasaki *et al.*, 2005). Aliquots (1 mL) from enrichment broth were transferred to microfuge tube and the cells were collected by centrifugation (15,000×*g*, 5 minutes). Briefly, the cells were resuspended in 200 μL of enzyme solution containing 1 mg/mL each of acromopeptidase and lysozyme in TE buffer. After incubation for 1 hour at 37°C, the solution was mixed with 300 μL of 4 M GuSCN containing 2% (w/v) Tween 20. A portion (400 μL) of the supernatant was transferred to a new tube containing 400 μL of 100% isopropanol. After mixing, the mixture was centrifuged for 10 minutes at 15,000×*g*, and the resulting DNA pellet was rinsed with 75% isopropanol. The pellet was then dissolved in 160 μL of distilled water by heating for 3 minutes at 70°C. Prior to use, the template DNA solution was centrifuged for 5 minutes at 15,000×*g* to further remove water-insoluble impurities. Two microliters of the solution was used as template for the PCR. The multiplex PCR amplification and conditions were as described previously (Kawasaki *et al.*, 2005). PCR products were analyzed by 2.5% agarose gel electrophoresis, and the expected size of the PCR products for *Salmonella*, *L. monocytogenes*, and *E. coli* O157:H7 were 375, 234, and 120 bp, respectively. The schematic representation of detection procedure is shown in Fig. 1.

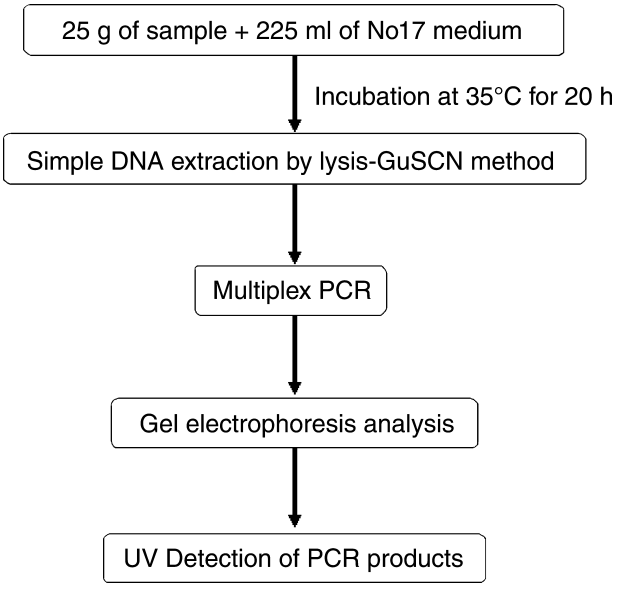

FIG 1. The scheme of multiplex polymerase chain reaction (PCR) assay for detection of *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes*.

Table 1. Comparison of Enrichment Times Using No17 Medium for Detection of *E. Coli* O157:H7, *Salmonella* Enteritidis, and *L. Monocytogenes* by Multiplex Polymerase Chain Reaction Assay

	Incubation time (h)		
Sample	*16*	*20*	*24*
Meat	+	+	+
Cabbage	+	+	+
Salmon	−[a]	+	+
Raw egg	+	+	+
Milk	+	+	+
Fresh cheese	+	+	+
Raw ham	−[a]	+	+

Each pathogen was inoculated at 5 colony-forming units per 25 g of sample.
[a]Only *L. monocytogenes* was not detectable.

Multiplex PCR evaluation in spiked food samples

Seven food samples (listed in Table 1) for the estimation of minimum enrichment time and the other 44 various food samples (listed in Table 2) for spike study, were purchased from local stores and were immediately transported in insulated coolers at 4°C to the laboratory for inoculation and analysis on the same day. For total bacterial count determinations, 25 g of each sample were mixed with 225 mL of PBS and pummeled in a Stomacher for 2 minutes. Serial decimal dilutions of samples prepared in PBS were pour plated (1 mL) in duplicate onto TSA. The plates were then incubated at 35°C for 18 hours before enumeration. For experiments in which the foods were inoculated, 100 μL of a mixed culture containing *Salmonella* Enteritidis, *L. monocytogenes*, and *E. coli* O157:H7 (0, 5, or 50 CFU of each organism) were mixed into 25 g of each food sample, and 225 mL of No17 medium was added. The mixture was then pummeled in a Stomacher for 2 minutes and incubated at 35°C for 24 hours without rotation. One milliliter of the culture broth was used for DNA extraction, followed by the multiplex PCR assay. When necessary, the enrichment culture was filtered through a 5-μm sterile micropore filter (Millipore, Bedford, MA) to trap most of the food materials before DNA extraction. For experiments examining the effect of frozen storage, spiked food samples were frozen at −20°C for periods of 2 weeks and 2 months, prior to DNA extraction and analysis by the multiplex PCR assay.

Multiplex PCR evaluation of naturally contaminated food samples

Seventy-seven food samples (listed in Table 3) were purchased from local stores, transported to the laboratory in an insulated container at 4°C, and analyzed on the same day. Twenty-five–gram samples were tested by the conventional methods described above and were mixed with 225 mL of No17 medium and incubated at 35°C for 24 hours. One milliliter of the enrichment was then subjected to the PCR assay after DNA extraction by the lysis-GuSCN method.

Results

Enrichment conditions

To determine the minimum enrichment incubation time using No17 medium, seven types of food samples (meat, cabbage, salmon, raw egg, milk, fresh cheese, and raw ham) inoculated with *Salmonella* Enteritidis, *L. monocytogenes*, and *E. coli* O157:H7 were used. As shown in Table 1, each pathogen was detectable by the multiplex PCR in all inoculated food samples with a sensitivity of 5 CFU of each pathogen per 25 g of inoculated samples after enrichment for 20 hours in No17 medium. Therefore, enrichment for 20 hours was used throughout this study.

Evaluation of the multiplex PCR assay with spiked food samples

The multiplex PCR assay for the detection of *Salmonella* Enteritidis, *L. monocytogenes*, and *E. coli* O157:H7 was evaluated with various food samples in which each pathogen was inoculated at 5–50 CFU per 25 g of sample (Table 2). Forty-four food samples were tested. The samples were meat (eight items), seafood (11 items), dairy products (13 items), vegetables (five items), and other foods (seven items). All pathogens were detectable with high sensitivity in 39 inoculated samples containing natural microflora at levels ranging from <10 to 10^8 CFU/g. Only *L. monocytogenes* was not detectable in four seafood samples (sardine, pond smelt, oyster,

Table 2. Summary of the Results of the Multiplex Polymerase Chain Reaction (PCR) Assay in Various Spiked Food Samples

Sample		*Multiplex PCR detection*			*Initial count (CFU/g)*
		Salmonella *Enteritidis*	L. monocytogenes	E. coli O157:H7	
Meat	Minced beef and pork	+	+	+	8.1×10^4
	Minced pork	+	+	+	7.6×10^3
	Pork liver	+	+	+	6.0×10^4
	Beef liver	+	+	+	1.1×10^4
Cutting meat	Raw beef with source	+	+	+	2.4×10^2
Processed meat	Raw ham	+	+	+	2.5×10^6
	Sausage (Zungenwurst)	+	+	+	5.8×10^7
	Meat paste	+	+	+	1.1×10^5
Seafood	Salmon	+	+	+	9.5×10^2
	Sardine	+	+[a]	+	4.9×10^2
	Pond smelt	+	+[a]	+	8.3×10^3
	Salmon roe (ikra)	+	+	+	2.0×10^3
	Raw shrimp (without shell)	+	+	+	1.2×10^4
	Frozen shrimp (without shell)	+	+	+	1.2×10^6
	Blacktiger (without shell)	+	+	+	1.2×10^4
	Squid (boil)	+	+	+	1.1×10^7
	Common mussel	+	+	+	3.8×10^3
	Clam	+	−	+	2.8×10^2
	Oyster	+	+[a]	+	1.8×10^2
Dairy products	Milk	+	+	+	<10
	Milk (low-temperature pasteurized)	+	+	+	<10
	Yogurt	+	+	+	<10
	Fresh cream	+	+	+	6.2×10^2
	Ice cream	+	+	+	<10
	Cream cheese	+	+	+	<10
	Cheese (Brie de Meaaux)	+	+	+	2.3×10^8
	Cheese (Cravanzina)	+	+	+	4.2×10^7
	Blue cheese (Bleu de Gex)	+	+	+	3.6×10^8
	Brie cheese	+	+	+	1.1×10^6
	Gouda cheese	+	+	+	3.3×10^3
	Mozzarella cheese	+	+	+	1.8×10^5
	Cultured butter	+	+	+	8.3×10^4
Egg	Egg	+	+	+	<10
Vegetable	Cabbage	+	+	+	4.7×10^4
	Spinach	+	+	+	6.3×10^6
	White radish sprouts	+	+	+	1.7×10^8
	Coleslaw	+	+	+	5.4×10^5
	Lettuce	+	+	+	3.0×10^2
Vinegared food	Seaweed	+	+	+	<10
Fermented food	Fermented soybeans (Natto)	+	+	+	5.8×10^8
Sweets	Milk chocolate (powder)	+[a]	+[a]	+[a]	3.0×10^1
	Milk chocolate (drink)	+	+	+	2.0×10^1
	Rice cake covered with bean jam	+	+	+	2.0×10^1
Condiment	Mayonnaise	+	+	+	<10

Each pathogen was inoculated at 5–50 colony-forming units (CFU) per 25 g of sample.
[a]Positive result was obtained by filter treatment of enrichment culture before DNA extraction.

Table 3. Results Obtained with the Multiplex Polymerase Chain Reaction (PCR) and Conventional Culture Methods from the Retail Food Samples

Sample		No. of samples	Salmonella *spp.*		L. monocytogenes		E. coli O157:H7	
			PCR	*Conventional*	*PCR*	*Conventional*	*PCR*	*Conventional*
Chicken	Breast	5	0	0	0	0	0	0
	Dark meat	9	3	2	4	2	0	0
	White meat	4	2	2	0	2	0	0
	Intestine	6	4	1	3	2	0	0
	Others	10	3	4	3	4	0	0
	Processed meat	3	0	0	1	1	0	0
Pork	Raw meat	6	0	0	1	1	0	0
	Intestine	3	0	0	0	0	1[a]	0
	Processed meat	5	1	0	0	1	0	0
Beef	Raw meat	5	0	0	1	1	0	0
	Processed meat	2	0	0	1	0	0	0
Pork and beef	Minced	3	0	0	1	0	0	0
Seafood	Fish	1	0	0	0	0	0	0
	Shellfish	1	0	0	0	0	0	0
	Processed	3	0	0	0	0	0	0
Salad		4	0	0	0	0	0	0
Vegetable		3	0	0	0	0	0	0
Cheese		1	0	0	0	0	0	0
Cake		3	0	0	0	0	0	0
Total		77	13	9	15	14	1	0

[a]*E. coli* O55 was isolated by the culture method.

and clam) by the multiplex PCR assay. In these samples, except for clam, *L. monocytogenes* was detected following filtration of the enrichment culture before performing DNA extraction. In milk chocolate powder, none of these pathogens were detectable by the multiplex PCR assay, but the detection sensitivity for the three pathogens was restored when the samples underwent filtration prior to DNA extraction.

Evaluation of the multiplex PCR assay with retail food samples

To evaluate the practical use of this multiplex PCR method for detecting the three pathogens in foods, results using the conventional culture method were compared to the PCR assay using 77 commercial food samples (Table 3). For *E. coli* O157:H7, one sample (pork intestine) was found positive using the multiplex PCR method, but this *E. coli* isolate was confirmed to be *E. coli* O55 by the culture method and serologically using O55 antisera (Denka Seiken). Of the 77 food samples, 13 were positive for *Salmonella* spp. by the multiplex PCR method, but only nine samples were positive by the conventional method. For *L. monocytogenes*, 15 samples were positive by the multiplex PCR assay in comparison to 14 samples by the conventional method.

Evaluation of the multiplex PCR assay with spiked frozen food samples

The results of detection of the pathogens from samples of meat, cabbage, salmon, raw egg, milk, fresh cheese, and raw ham stored at −20°C for periods of 2 weeks and 2 months are shown in Tables 4 and 5. The detection rate for each pathogen by multiplex PCR was higher than that of the conventional culture methods in all the post-storage frozen samples. The detection rate by both methods decreased in samples stored for 2 months at −20°C compared to those stored for 2 weeks. This was evident particularly for *Salmonellla* Enteritidis since detection by the culture method declined considerably after frozen storage. The detection rate for each pathogen by multiplex PCR was greater than 75% for all food samples after frozen storage for 2 months.

Discussion

In a previous study, we developed an enrichment medium (No17) that allows simulta-

Table 4. Results Obtained with the Multiplex Polymerase Chain Reaction (PCR) and Conventional Culture Methods in the Frozen Food Samples After Storage for 2 Weeks at −20°C

		Salmonella *Enteritidis*		L. monocytogenes		E. coli O157:H7	
Frozen samples	*No. of samples*	*PCR*	*Conventional*	*PCR*	*Conventional*	*PCR*	*Conventional*
Meat	4	4	0	4	4	4	4
Cabbage	4	3	1	3	3	3	2
Salmon	4	4	4	4	4	4	3
Raw egg	4	4	4	4	4	4	4
Milk	4	4	3	4	4	4	2
Fresh cheese	4	4	1	4	3	3	3
Raw ham	4	4	0	4	4	4	2
Total	28	27	13	27	26	26	20

Each pathogen was inoculated at 5–50 colony-forming units per 25 g of sample.

neous growth of *Salmonella* Enteritidis, *L. monocytogenes*, and *E. coli* O157:H7 for subsequent detection of each pathogen using a multiplex PCR assay with similar sensitivity. In spiked pork samples, a detection sensitivity of one cell of each pathogen per 25 g of inoculated samples was obtained after enrichment for 24 h in this medium (Kawasaki *et al*., 2005). In the current study, we have determined that the enrichment time can be shortened to 20 hours using this method to obtain a similar sensitivity. There were no significant differences in sensitivity between pure culture and inoculated food samples.

The practical application of this multiplex PCR assay was tested using 44 different food samples. *Salmonella* Enteritidis, *L. monocytogenes*, and *E. coli* O157:H7 were detected in almost all food samples inoculated at 5 CFU per 25 g. Only in clam samples could the detection sensitivity not be restored by filtration of the enrichment. This might be due to its high glycogen content, which may inhibit the PCR due to carryover. Renault *et al*. (2000) also described how the presence of unidentified substances in oyster tissues inhibited the PCR. Wolffs *et al*. (2006) described a sample preparation technique using a two-step filtration method for PCR detection of *Salmonella* from chicken rinse and irrigation water samples. They described a recovery protocol combining the removal of large crude food particles by a >40-μm sterile filter and the capture of the target cell on a 0.22-μm sterile filter. However, it is difficult to use a filtration capturing technique with a 0.22-μm sterile filter with foods, such as chicken, ground beef, or milk because particles would readily clog the filter. In another study, a flotation method based on density gradient centrifugation was used for cell separation from a food matrix (Wolffs *et al*., 2004; Fukushima *et al*., 2007). However, this procedure requires many steps and careful transferring of the upper layer so that DNA can be extracted from the solution

Table 5. Results Obtained with the Multiplex Polymerase Chain Reaction (PCR) and Conventional Culture Methods in the Frozen Food Samples After Storage for 2 Months at −20°C

		Salmonella *Enteritidis*		L. monocytogenes		E. coli O157:H7	
Frozen samples	*No. of samples*	*PCR*	*Conventional*	*PCR*	*Conventional*	*PCR*	*Conventional*
Meat	4	4	0	4	3	4	4
Cabbage	4	2	0	2	2	2	0
Salmon	4	4	3	4	2	4	4
Raw egg	4	4	2	3	4	4	4
Milk	4	4	1	4	4	3	2
Fresh cheese	4	2	0	3	3	2	2
Raw ham	4	4	0	4	4	4	2
Total	28	24	6	24	22	23	18

Each pathogen was inoculated at 5–50 colony-forming units per 25 g of sample.

after the separation protocol. Therefore, neither the two-step filtration nor the flotation method is practical for detecting pathogens from a large number of samples. In our DNA extraction protocol, pretreatment by filtration with a 5-μm sterile filter removed almost all crude food particles that would otherwise inhibit the PCR. This DNA extraction protocol can be used as a routine method for detecting pathogens from almost all food samples.

In the naturally contaminated samples, excellent agreement was obtained between the results of multiplex PCR and conventional culture methods, indicating that multiplex PCR is a reliable and useful method for rapid screening of food samples for contamination by these pathogens. Only one sample gave a positive result for *E. coli* O157:H7 with the multiplex PCR method. A primer set targeting the *E. coli* O157:H7 *eae* gene was used in this multiplex PCR assay. Primers targeting this gene were reported to detect enteropathogenic isolates belonging to serogroup O55 in addition to *E. coli* O157:H7 due to the similarity of this gene in the two organisms (Sharma *et al*., 1999).

The detection rate of the multiplex PCR method was higher than that of the conventional culture method for all the frozen samples. This may be due to the ability of No17 medium to allow recovery of injured cells, since this medium does not contain antimicrobial chemicals. Uyttendaele *et al*. (1998) described the necessity of sufficient enrichment time for PCR detection of cold-stressed enterohemorrhagic *E. coli*. They showed that when enterohemorrhagic *E. coli* strains were inoculated at levels of less than 10 CFU in 25 g of ground beef, PCR results were negative with a 24-hour enrichment period after storage for greater than 1 week at −20°C. However, they used *Escherichia coli* (EC) broth for the enrichment step, and clearly, the presence of certain components, such as bile salts, can prevent recovery of injured cells and may require a longer enrichment phase prior to PCR detection. Optimization of methods for detection of different pathogens is necessary, in terms of both the DNA extraction and PCR amplification steps. Furthermore, different pathogens may require different enrichment media and procedures. However, we have overcome some of these limitations through the use of a nonselective enrichment step using No17 medium followed by a multiplex PCR assay for simultaneous detection of three important pathogens, reducing the total analysis time without loss of sensitivity.

In conclusion, the multiplex PCR assay described in this study for the detection of *Salmonella* spp., *L. monocytogenes*, and *E. coli* O157:H7 is capable of detecting as low as 5 CFU per 25 g of each pathogen in more than 40 types of food samples after enrichment in No17 broth. Furthermore, the detection rate in inoculated frozen samples exceeded that of the conventional culture methods. This multiplex assay will be valuable as a screening method for foods contaminated with these pathogens, and will also be useful for identifying the sources of foodborne outbreaks.

Acknowledgments

This work was supported in part by a grant (Research project for ensuring food safety from farm to table, D1-7107) from the Ministry of Agriculture, Forestry, and Fisheries of Japan.

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

Disclosure Statement

No competing financial interests exist.

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